
بسم الله الرحمن الرحيم
In the name of God, the Beneficent, the Merciful

Glaucoma and Gene Therapy

Presented by: Sajjad Hamze Mostafavi

M.Sc Student of medical biotechnology

School of Paramedical Sciences

Qazvin University of Medical Sciences

Under supervision of: Dr. Ahmadpour Yazdi

Content:

Part 1: Glaucoma, types and Prevalence

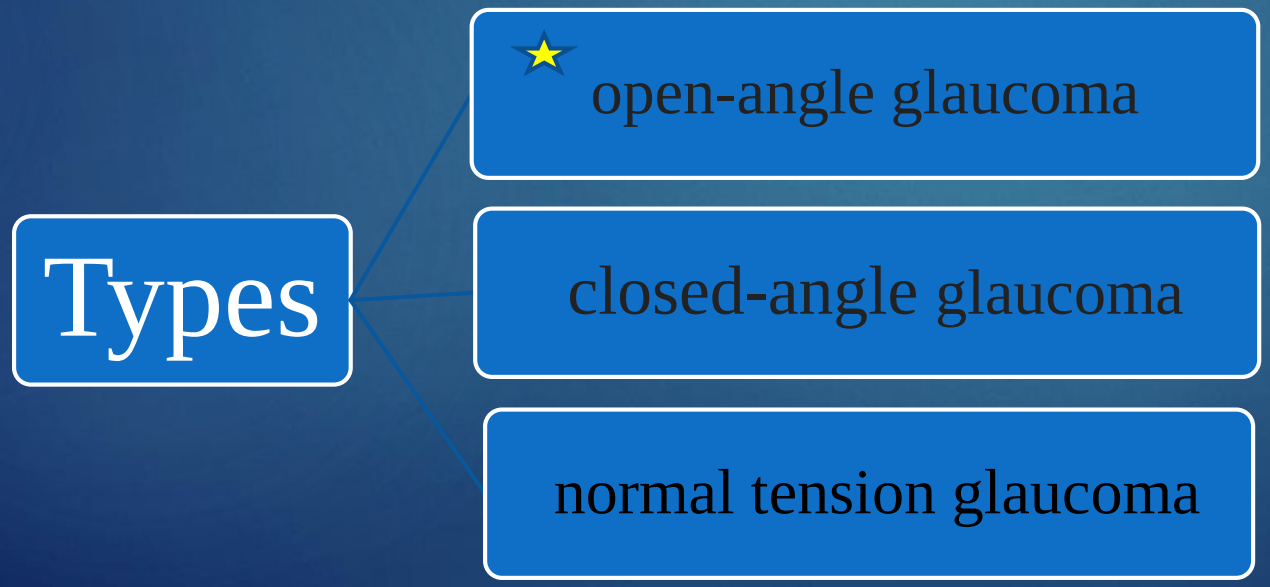
Part 2: Common treatments, side effects and challenges

Part 3: Gene Therapy and Limitations

Part 1

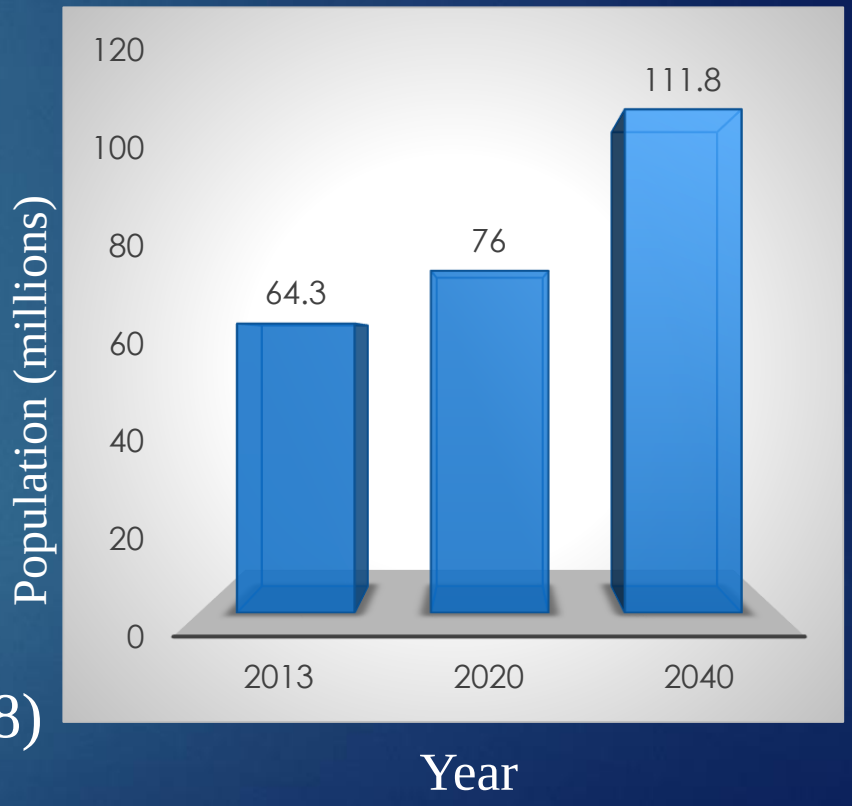
What is glaucoma?

- a potentially devastating disease of the eye
- Progressive and irreversible degeneration of retinal ganglion cells(**RGC**)
- affects mainly people over 40 years of age



Global Prevalence of Glaucoma

- One of the main causes of global irreversible blindness
- The global prevalence of glaucoma(40-80) ⟶ 3.54%
- POAG in Africa ⟶ 4.20%
- PACG in Asia ⟶ 1.09%
- The ratio of men to women for POAG (OR, 1.36)
- The ratio urban areas to rural areas for POAG (OR, 1.58)



Risk factors

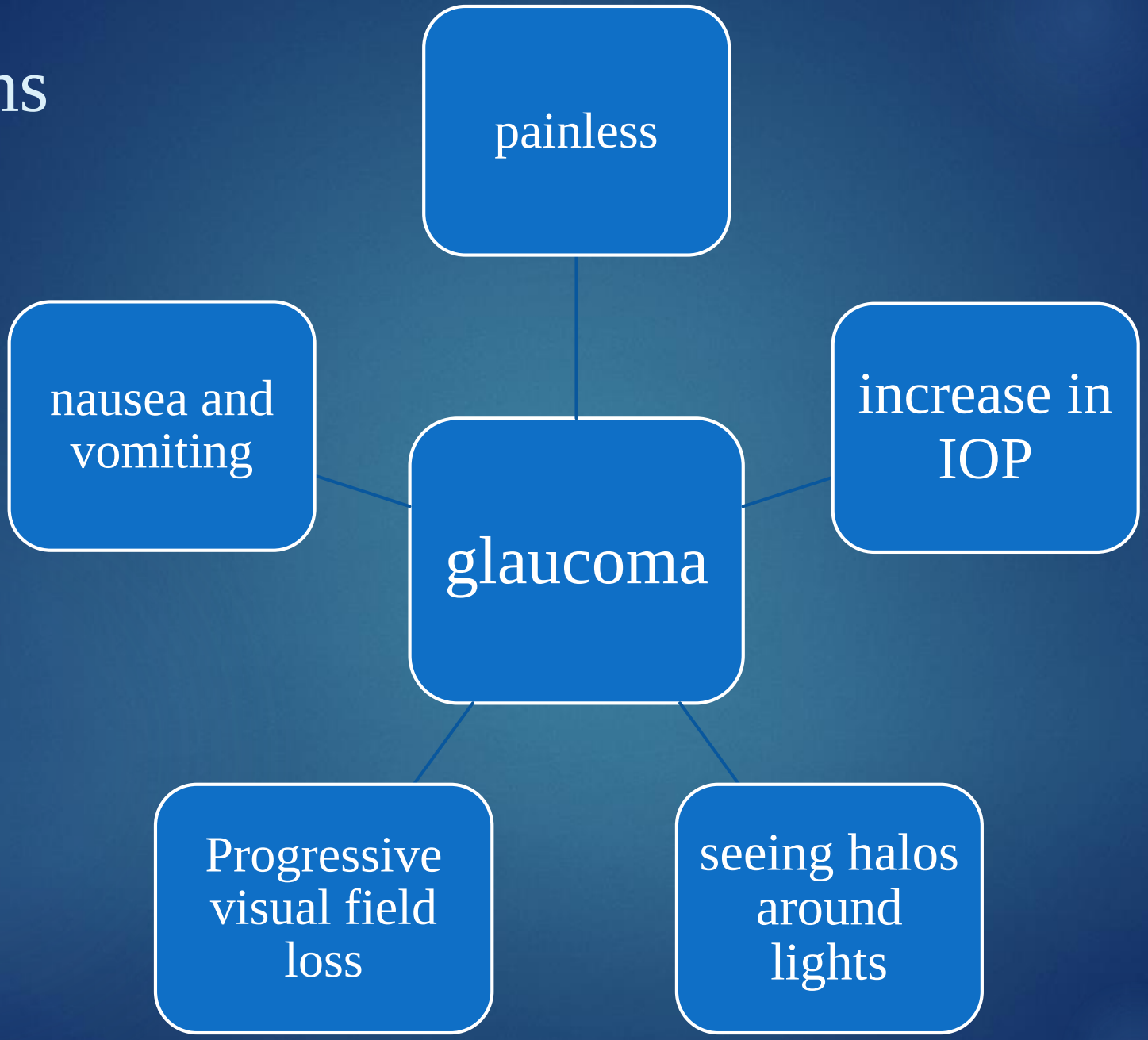
Box 1
Risk factors for primary open-angle glaucoma

- Elevated IOP **(intraocular pressure)**
- Older age
- Thinner central cornea **(corneal thickness)**
- African American race
- Family history

Box 2
Risk factors for angle-closure glaucoma

- Older age
- Female gender
- Asian ethnicity
- Hyperopia

symptoms


painless
nausea and vomiting
glaucoma
increase in IOP
Progressive visual field loss
seeing halos around lights

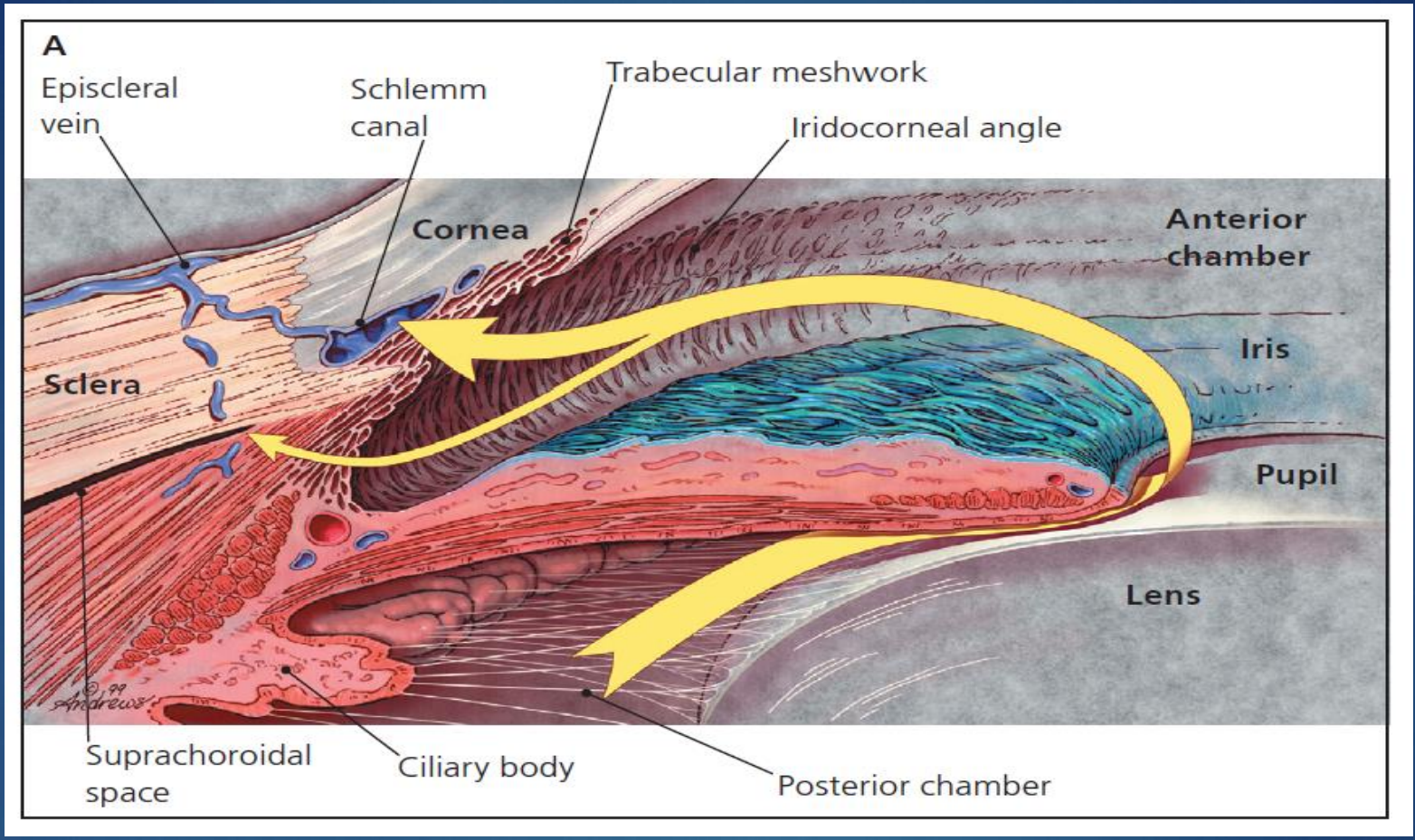
Definition of intraocular pressure (IOP)

- a balance of aqueous humor production and drainage it.

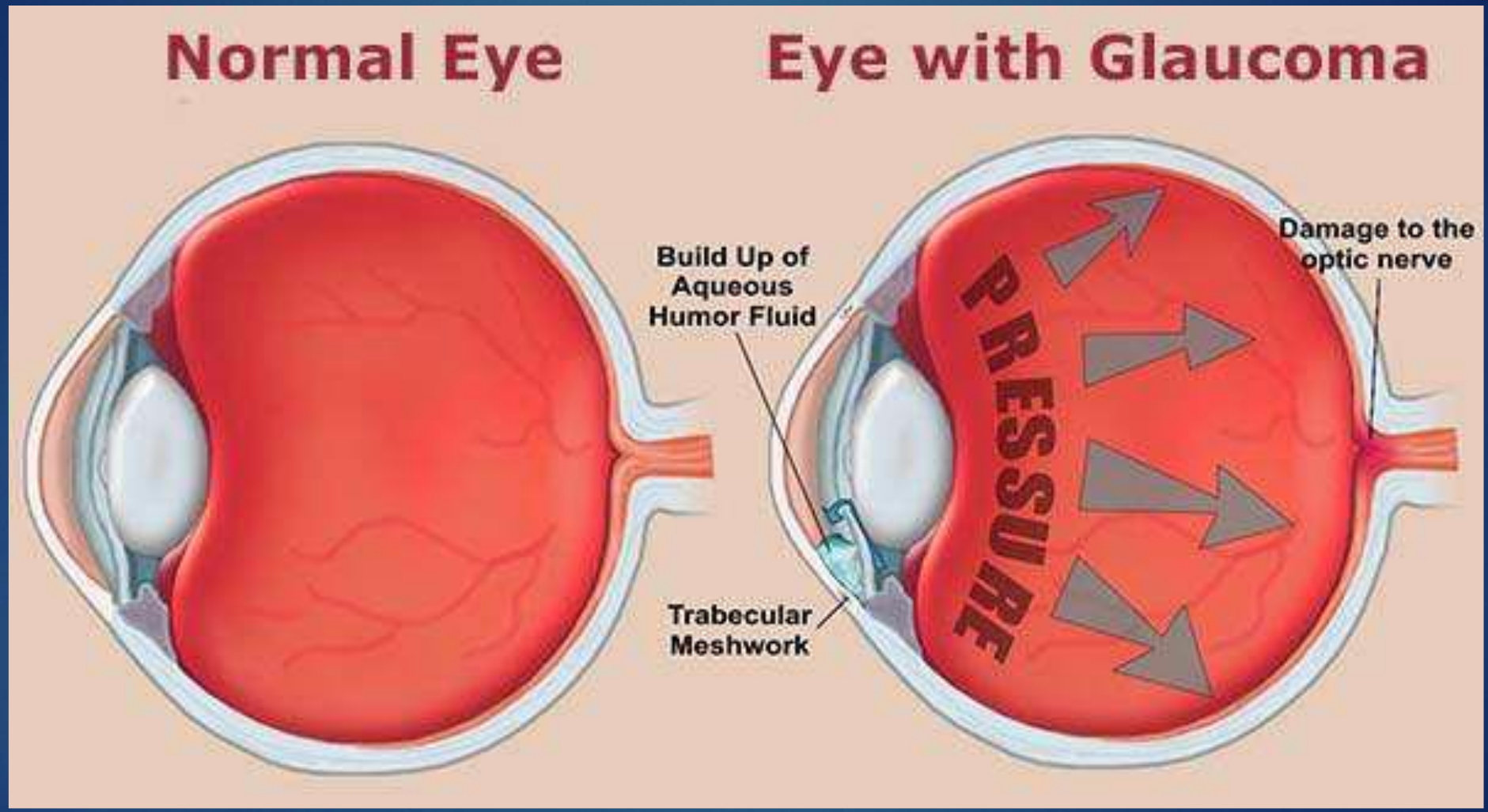
Possible causes leading to an increase in IOP

- side-effects from medications
- genetic factors
- anatomical problems
- inflammation of the eye

aqueous humor Normal flow



outflow through trabecular meshwork (large arrow) and uveoscleral routes (small arrow) and related anatomy. Most aqueous flow is through the trabecular meshwork. Each pathway is drained by the eye's venous circulation


Normal Eye
Eye with Glaucoma
Build Up of Aqueous Humor Fluid
Damage to the optic nerve
PRESSURE
Trabecular Meshwork

Disease progression

Diagnosis

Test Name	Test explains about
Tonometry	Inner eye pressure
Gonioscopy	Angle Examination
Ophthalmoscopy	Dilated eye examination shape and color of the optic nerve
Perimetry or Visual field test	Complete field of vision

- Normal → 10-20 mmHg
- In POAG → > 22 mmHg
- In PACG → > 30 mmHg

https://www.digitaleyecenter.com

Part 2: Glaucoma treatment

based solely on lowering the intraocular pressure (IOP)

The standard clinical treatment:

medicines

- multiple different eye drops administered several times per day
- patient compliance is a challenge
- progressive visual loss even after IOP reduction

laser treatment and surgery

- Have risks
- often require a combinational approach supplementing
- additive topical therapies throughout a patient's life

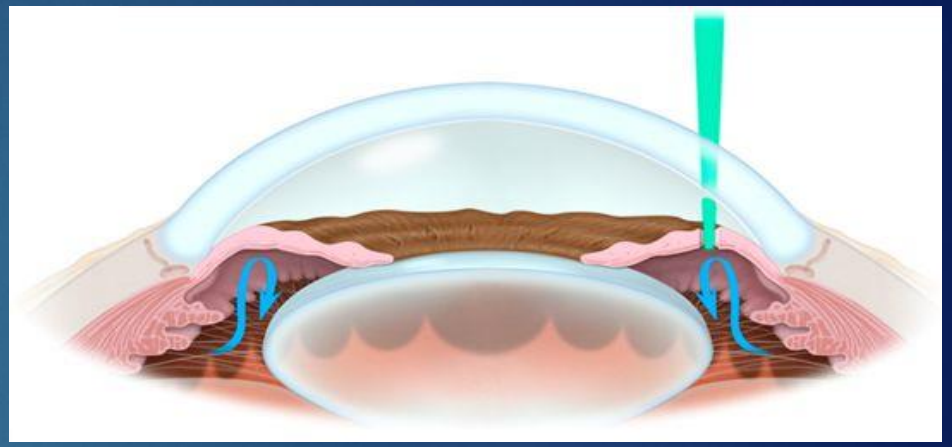
medicines

Medication class	Mechanism of action	Drug names
Alpha-adrenergic agonists	aqueous humor production ↓	Apraclonidine (Iopidine), brimonidine (Alphagan)
Beta blockers	aqueous humor production ↓	Betaxolol (Betoptic), levobunolol(Betagan)
Carbonic anhydrase inhibitors	aqueous humor production ↓	Brinzolamide (Azopt), dorzolamide (Trusopt)
Cholinergics	Outflow through trabecular meshwork ↑	Pilocarpine
Prostaglandin analogues*	Outflow through trabecular meshwork ↑	Bimatoprost (Lumigan), latanoprost (Xalatan)

laser

aims: improving aqueous humor drainage

- argon laser trabeculoplasty (ALT)
- leading to opening of meshwork
- selective laser trabeculoplasty (SLT).
- stimulating drainage in the trabecular meshwork
- SLT can be repeated three or four times
- Laser Peripheral Iridectomy
- most for closed-angle glaucoma



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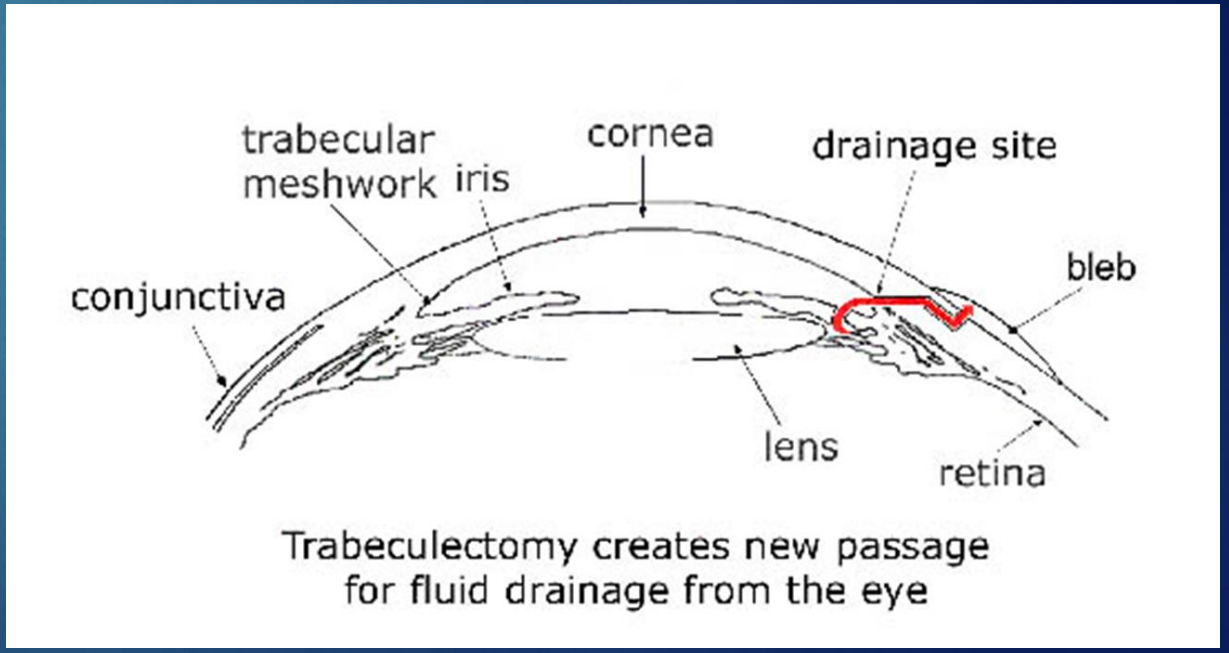
surgery

- Both laser and conventional surgeries are a temporary solution
- often used when patients are refractory to medical and/or laser therapies
- most common method ⟶ trabeculectomy
- Efficiency ⟶ Often up to 2 years
- The risk of intraocular infections



Trabeculectomy creates new passage for fluid drainage from the eye

http://www.djo.harvard.edu

Part 3: Gene Therapy in glaucoma

Gene therapy

- an approach of treating diseases
  - by modifying the expressions of individual's genes
  - by correction of abnormal genes
- This can be accomplished by:
  - Replacing a mutated gene that causes disease with a healthy copy of the gene.
  - Inactivating, or "knocking out", a mutated gene that is functioning improperly.
  - Introducing a new gene into the body to help fight a disease.

TYPES OF GENE THERAPY:

Classified into two types

- GERM LINE GENE THERAPY:

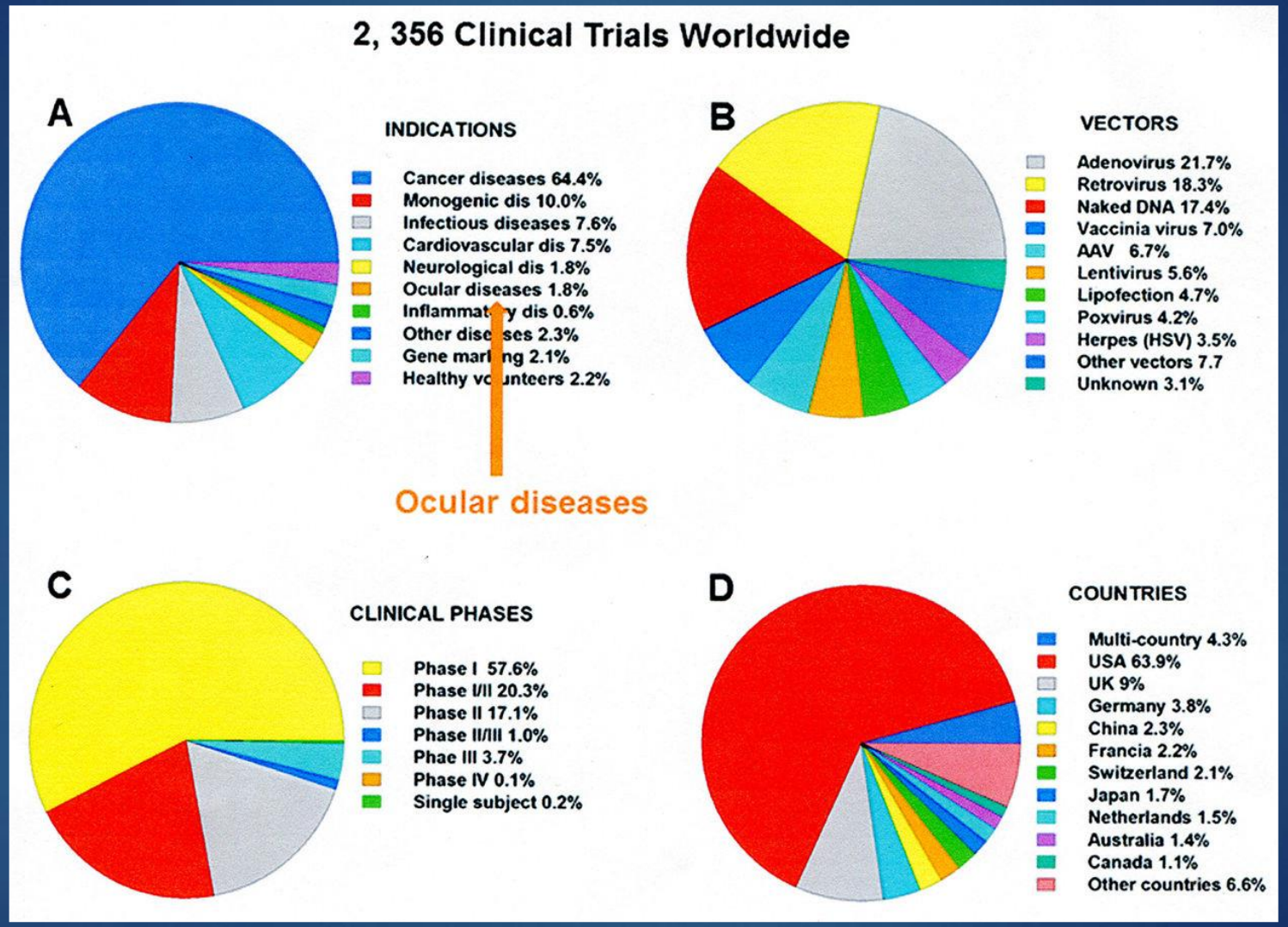
  Germ cells, i.e., sperm or eggs are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Heritable

- SOMATIC GENE THERAPY:

  The therapeutic genes are transferred into the somatic cells. Inheritable

- The eye is at the forefront of the application of gene therapy techniques to medicine.
- vectors delivered to the eye are relatively isolated from the rest of the body
- The size and ease of access to the eye is also favourable
- allowing small volumes of drug to be precisely delivered

The position of eye diseases gene therapy



Data obtained from the Journal of Gene Medicine database http://www.abedia.com/wiley/index.html updated in February 2016.

Review Article

Progress in Gene Therapy to Prevent Retinal Ganglion Cell Loss in Glaucoma and Leber's Hereditary Optic Neuropathy

Sara E. Ratican,[1,2] Andrew Osborne,[1] and Keith R. Martin[1,3,4,5]

[1]*John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK*
[2]*Geisel School of Medicine, Dartmouth College, Hanover, NH, USA*
[3]*Eye Department, Addenbrooke's Hospital, Cambridge, UK*
[4]*Cambridge NIHR Biomedical Research Centre, Cambridge, UK*
[5]*Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK*

Correspondence should be addressed to Keith R. Martin; krgm2@cam.ac.uk

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The eye is at the forefront of the application of gene therapy techniques to medicine. In the United States, a gene therapy treatment for Leber's congenital amaurosis, a rare inherited retinal disease, recently became the first gene therapy to be approved by the FDA for the treatment of disease caused by mutations in a specific gene. Phase III clinical trials of gene therapy for other single-gene defect diseases of the retina and optic nerve are also currently underway. However, for optic nerve diseases not caused by single-gene defects, gene therapy strategies are likely to focus on slowing or preventing neuronal death through the expression of neuroprotective agents. In addition to these strategies, there has also been recent interest in the potential use of precise genome editing techniques to treat ocular disease. This review focuses on recent developments in gene therapy techniques for the treatment of glaucoma and Leber's hereditary optic neuropathy (LHON). We discuss recent successes in clinical trials for the treatment of LHON using gene supplementation therapy, promising neuroprotective strategies that have been employed in animal models of glaucoma and the potential use of genome editing techniques in treating optic nerve disease.

The first gene therapy to be approved by the FDA for the eye diseases.

- It is one of the first people to undergo gene therapy for the treatment of genetic blindness
- The person with it is only able to observe the halo of light
- The cause is mutation in RPE65 gene
- Luxturna is injected into the eye
- It is suitable for children right now, because in the retina the baby is developing

Source: Dailymail

- Due to the challenges and side effects of current methods
- need for a single injection directly into the eye
- lead to long-lasting or permanent beneficial outcomes

The Pathway From Genes to Gene Therapy in Glaucoma: A Review of Possibilities for Using Genes as Glaucoma Drugs

Teresa Borrás, PhD
Department of Ophthalmology, University of North Carolina School of Medicine, Chapel Hill, North Carolina

Abstract

Treatment of diseases with gene therapy is advancing rapidly. The use of gene therapy has expanded from the original concept of replacing the mutated gene causing the disease to the use of genes to control nonphysiological levels of expression or to modify pathways known to affect the disease. Genes offer numerous advantages over conventional drugs. They have longer duration of action and are more specific. Genes can be delivered to the target site by naked DNA, cells, nonviral, and viral vectors. The enormous progress of the past decade in molecular biology and delivery systems has provided ways for targeting genes to the intended cell/tissue and safe, long-term vectors. The eye is an ideal organ for gene therapy. It is easily accessible and it is an immune-privileged site. Currently, there are clinical trials for diseases affecting practically every tissue of the eye, including those to restore vision in patients with Leber congenital amaurosis. However, the number of eye trials compared with those for systemic diseases is quite low (1.8%). Nevertheless, judging by the vast amount of ongoing preclinical studies, it is expected that such number will increase considerably in the near future. One area of great need for eye gene therapy is glaucoma, where a long-term gene drug would eliminate daily applications and compliance issues. Here, we review the current state of gene therapy for glaucoma and the possibilities for treating the trabecular meshwork to lower intraocular pressure and the retinal ganglion cells to protect them from neurodegeneration.

One area of great need for eye gene therapy is glaucoma, where a long-term gene drug would eliminate daily applications and compliance issues.

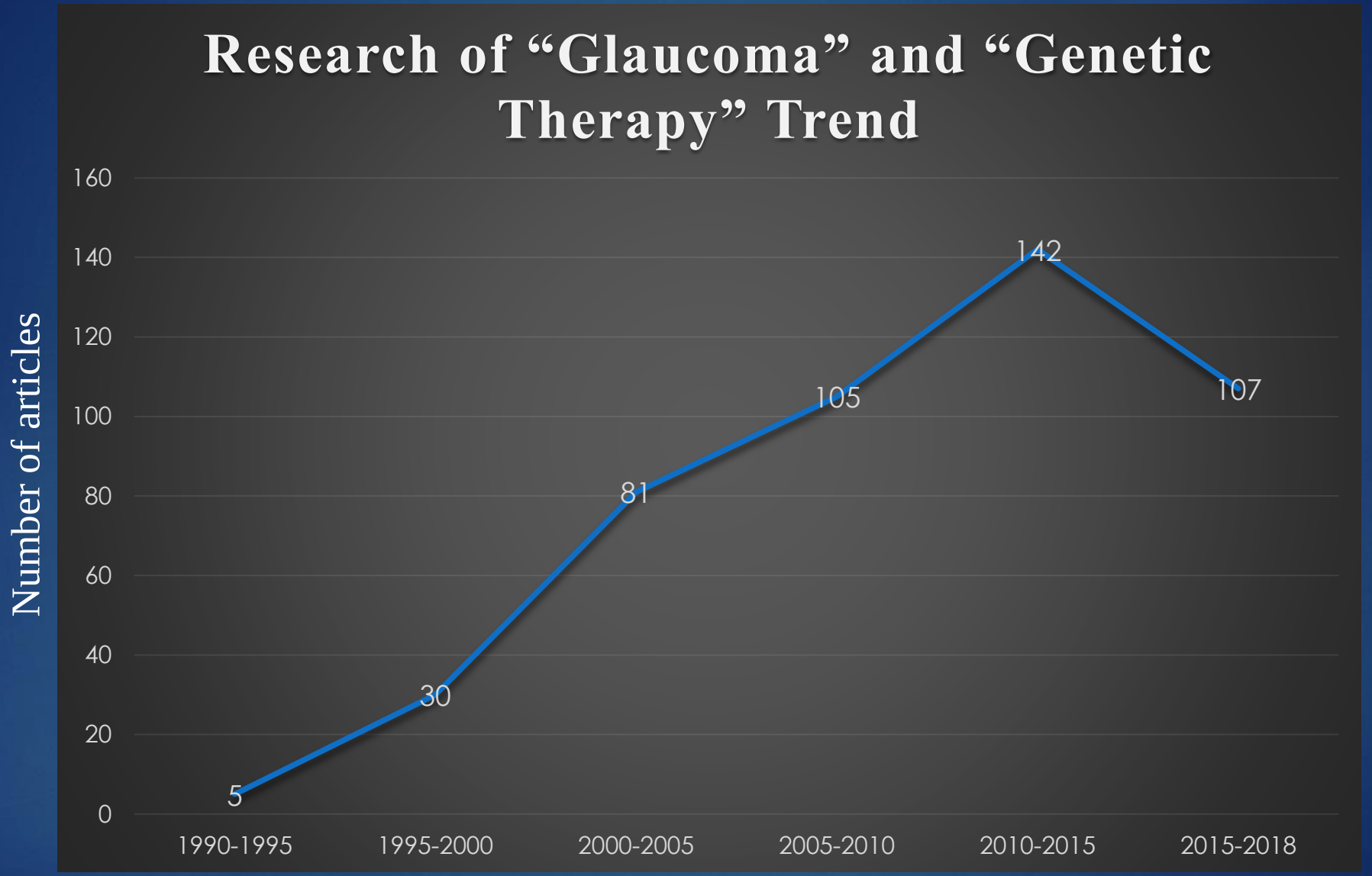

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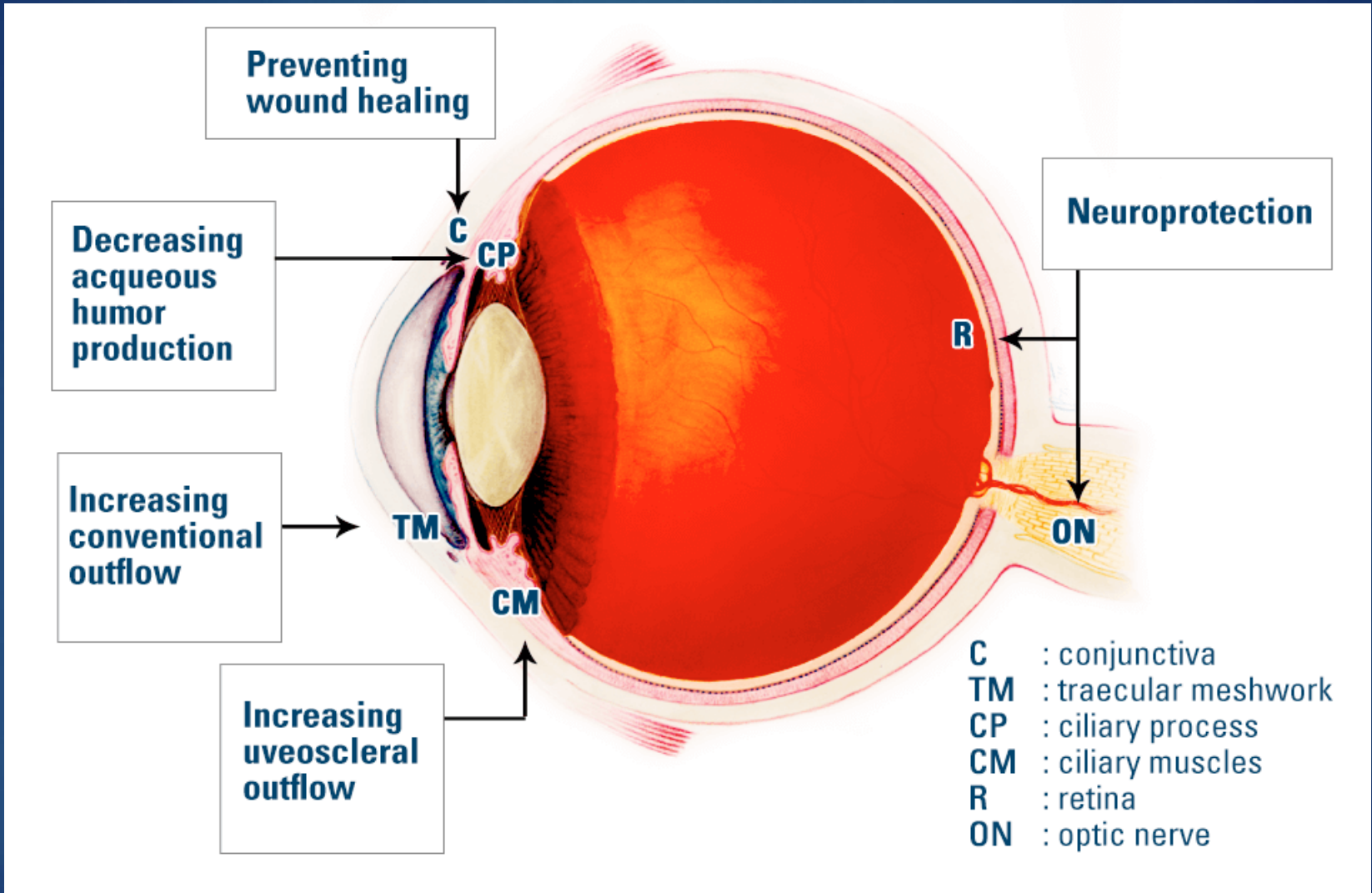
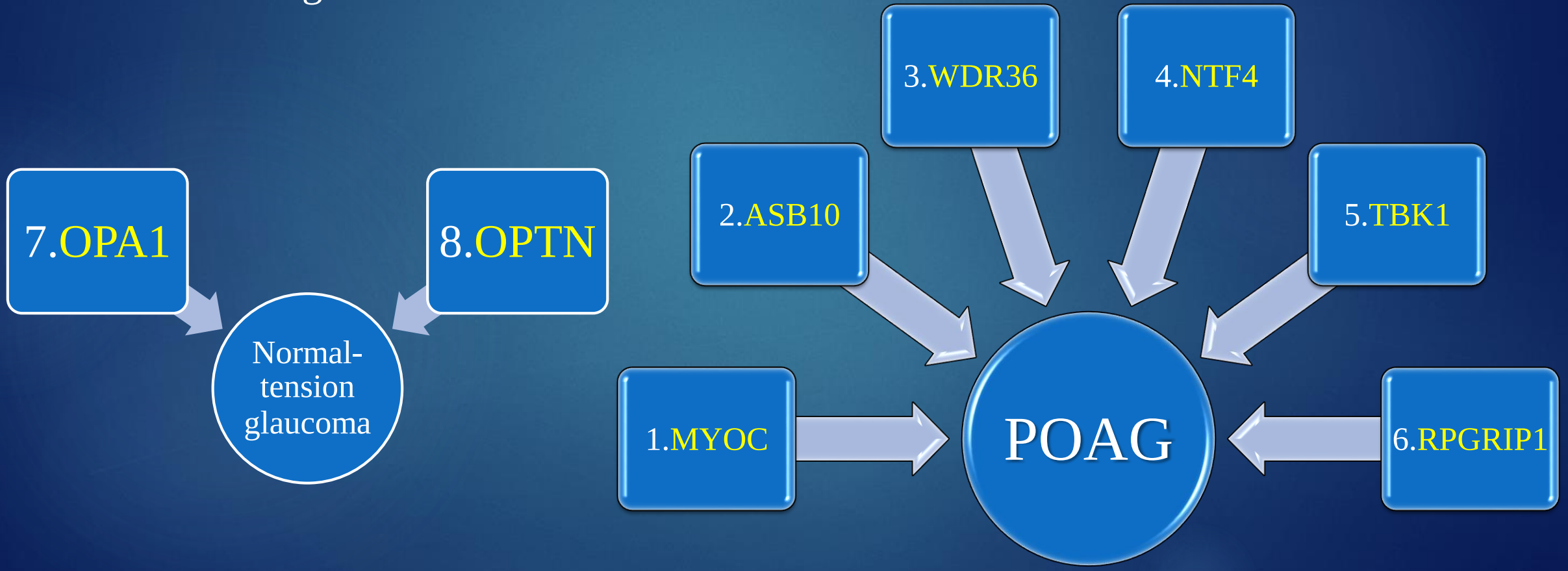
Diagram showing different ocular target tissues for glaucoma gene therapy.



Eye diagram adapted from National Eye Institute, National Institutes of Health

Glaucoma & genetics

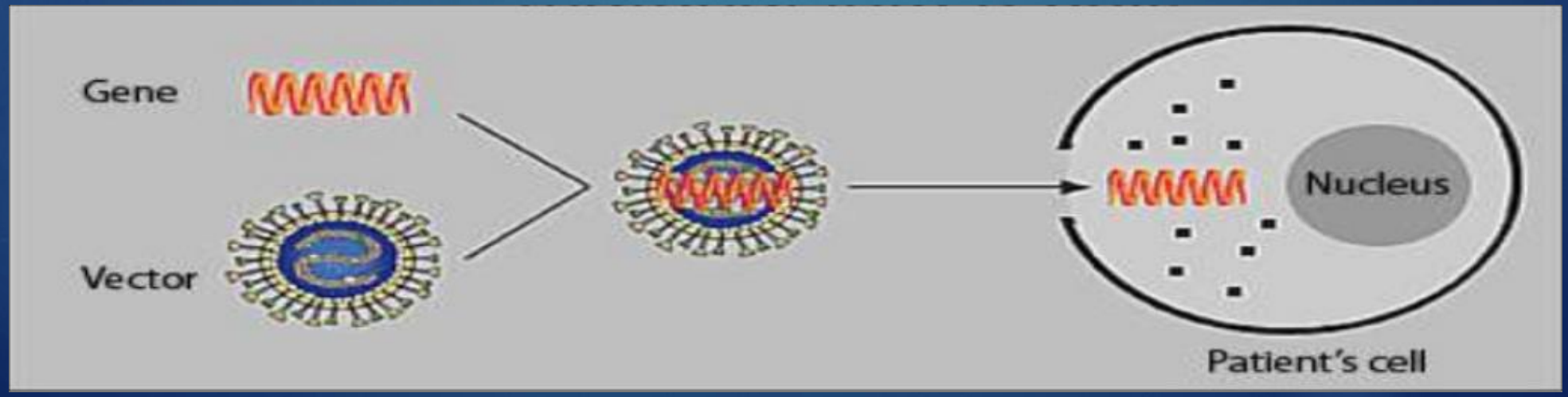
Glaucoma is associated with mutations in several genes:



	protein	gene	Gene location	characteristics
1	Myocilin(MYOC)	MYOC	1q24.3	• Mutations in MYOC are a major cause of glaucoma(POAG)
2	ankyrin repeat and SOCS box containing 10	ASB10	7q36.1	• is associated with POAG
3	WD repeat-containing protein 36	WDR36	5q22.1	• a member of the WD repeat protein family • The mutation causes POAG
4	NTF4	Neurotrophin4 (NT-4)	19q13.33	• a neurotrophic factor • signals through the TrkB receptor tyrosine kinase
5	TANK-binding kinase 1	TBK1	12q14.2	• Serine/threonine-protein kinase • this gene is similar to IKB kinases
6	X-linked retinitis pigmentosa GTPase regulator-interacting protein	RPGRIP1	14q11.2	• Defects in the gene result in glaucoma
7	Dynamin-like mitochondrial protein	*OPA1*	3q29	• prevents cell death • Mutations in this gene lead to loss in vision, hearing, muscle contraction
8	Optineurin	OPTN	10p13	• may play a role in normal-tension glaucoma and POAG

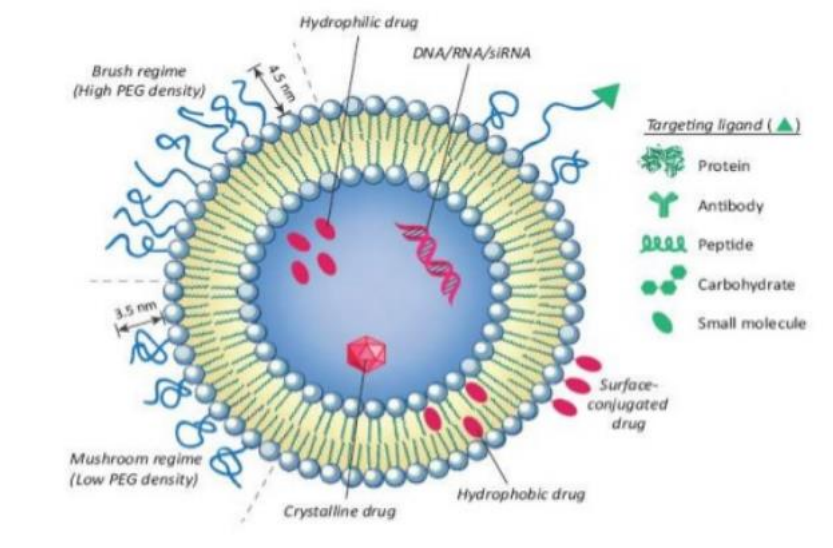
Vectors in Gene Therapy

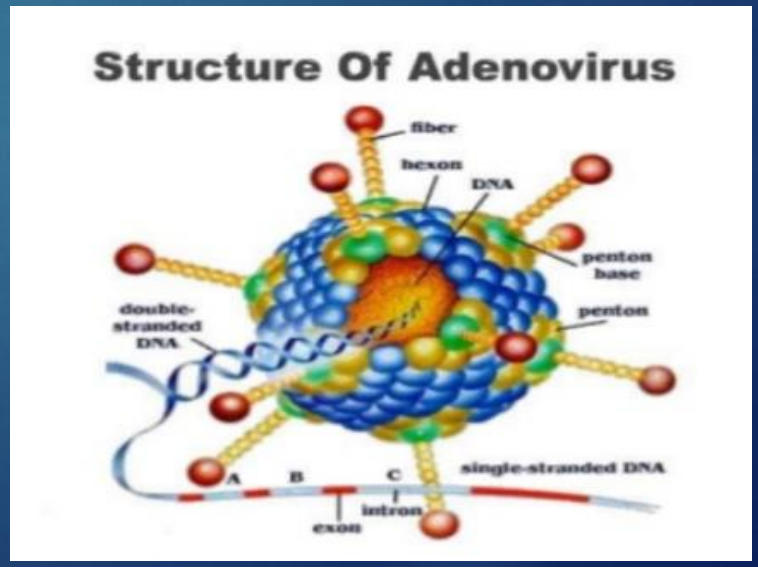
- To transfer the desired gene into a target cell, a carrier is required.
- Such vehicles of gene delivery are known as vectors.
- 2 main classes:
  - Viral vectors
  - Non viral vectors



Types of Gene Therapy Vectors

- Non-viral vectors
  - Naked DNA
  - Liposomes/DNA
  - Polymer/DNA complex (polyplex)
  - Liposome/Polymer/DNA
  - (lipopolyplex)
  - Metalic NPs
- Viral vectors
  - DNA viruses
    - Adenovirus
    - Adeno-Associated Virus(AAV)
    - Herpes Simplex Virus
  - RNA viruses
    - Retrovirus





Vector for Glaucoma Therapy

- a successful gene therapy in the eye need a vector system
- that leads to long, sustained levels of therapeutic gene expression within a select target cell
- minimal side effects
- This is particularly important for chronic, long-term pathologies such as glaucoma

- various vector systems are used for retinal gene therapy
- such as adenovirus, lentivirus, and nanoparticles
- recombinant adeno-associated viral vectors (AAVs) have proven the most promising
- a 4.7 kb single-stranded genome
- 4.4 kb of the viral genome is deleted
- vectors be packaged with a similar-sized foreign piece of DNA
- AAV2 is the most efficient serotype for RGC transduction

AAV difference with other vectors

	Lentivirus	AAV	Adenovirus
Diameter	90 nm	25 nm	100 nm
Genome	ssRNA	ssDNA	dsDNA
Packaging Capacity	~8kb	~5kb	~8kb*; ~30kb#
Tropism	Broad	Broad/Distinct†	Broad
Peak Expression	4-6 Days	2-4 Weeks	2-4 Days
Expression Duration	Long-Term	Long-Term	1-4 Weeks
Immune Response	Moderate	Mild	Strong

https://www.sciencedirect.com

AAV limitation

- small cargo capacity of AAV vectors for foreign DNA
- large genes are not suitable for use in a standard AAV vector
- dual vector and more recently triple vector approaches are being designed to overcome the coding capacity
- Splitting large genes into two halves and packing them into two independent AAV vectors
- this method is less effective than single AAV-mediated gene delivery

Promoter

- Promoter choice is also important for cell-specific expression
- lead to strong transgene expression within the targeted cell
- between several RGC-specific promoters many designed vectors employ:
    - CMV (Cytomegalovirus)
    - hybrid CMV early enhancer/chicken b-actin promoter (CAG)
- their Advantages small size and high levels of transgene expression

Promoters typically chosen for transduction of retinal ganglion cells (RGCs) within the eye.

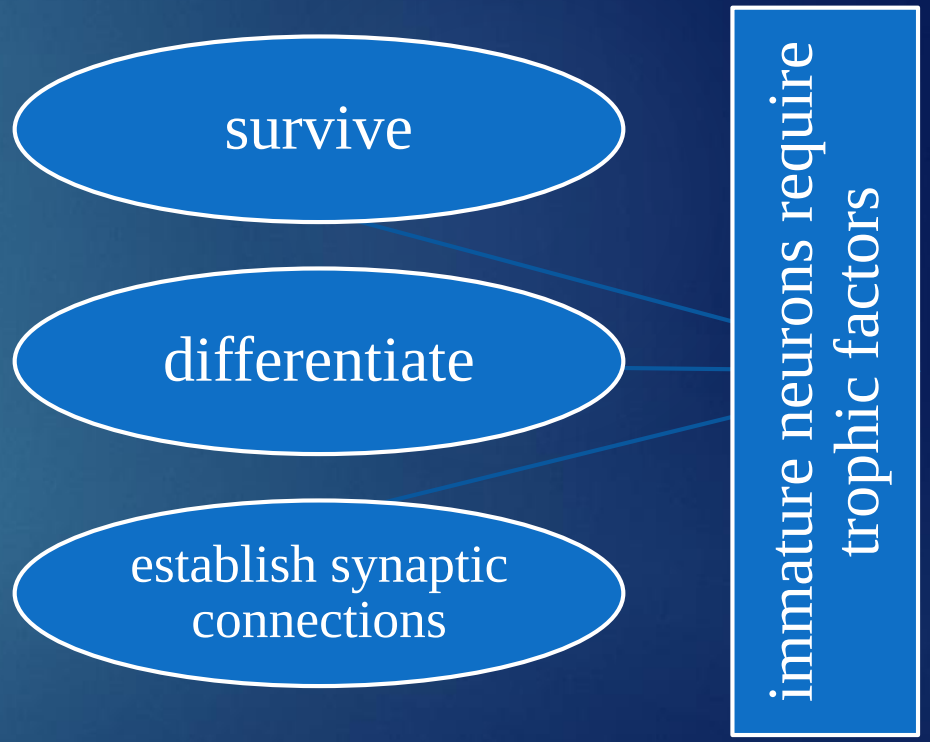
Promoter	Strength of expression in RGCs	Off-target labelling	Size (bp) % of AAV cargo
CMV	+++	Muller glia Amacrine cells Bipolar cells	(508–800) *(10–17%)*
CAG	++++	Muller glia Amacrine cells Bipolar cells	(584–1132) *(12–24%)*
SYN1	++	Amacrine cells	(400–469) *(8–10%)*
Nefh	+++		(2251) *(48%)*
Thy1	++		(6500) (exceeds limit)
Mcp-1	+++	injured cells	560 (mouse only) *(12%)*

- gene therapies for adult-onset glaucoma have focused primarily on neuroprotection
- involves slowing the loss of RGCs
- This can be accomplished in two ways:

  (1) by enhancing the activity of innate survival pathways in RGC

  (2) by inhibiting the progression of cell death

- Neurotrophic factors → promote neuron survival
- During development of the central nervous system
- To control these processes:
- some neurotrophic factors are expressed in limited quantities by target tissues
- only neurons exposed to optimal neurotrophic levels survive and establish synaptic connections



- in neurodegenerative diseases:
- Neurotrophic factors as potential neuroprotective factors
- brain-derived neurotrophic factor (BDNF)
- Ciliaryderived neurotrophic factor (CNTF)
- protect axotomized RGC and RGC in animal models of glaucoma

- The biological effects of neurotrophins are mediated by cell surface receptors
- BDNF acts by binding to the receptor tropomyosin-related kinase B (TrkB)
- stimulates multiple signalling pathways within RGC
  - extracellular signal-regulated kinases 1/2 (Erk 1/2)
  - the phosphatidylinositol-3 kinase (PI3K)/Akt

- Research has shown that intraocular injection of BDNF protein or AAV-mediated BDNF expression provides a robust but temporary neuroprotective effect on RGC after optic nerve transection or crush

- the Other approach in glaucoma gene therapy is inhibition the progression of RGC apoptosis
- Caspase activity is the final common element in the implementation of apoptosis in RGC
- caspase inhibitors an appealing prospect for neuroprotective glaucoma therapies

- Taking this approach, McKinnon et al:
  - injected an AAV-CAG vector expressing a caspase inhibitor
  - baculoviral IAP repeat-containing protein-4 (BIRC4)
  - one eye on rat models of glaucoma
  - BIRC4 was shown to significantly promote RGC survival
  - Despite the significant differences in IOP exposure among treatment groups

Rho associated kinase (ROCK) inhibitors:

- ROCK is a serine/threonine kinase
- Rho kinase inhibitors, such as Ripasudil work by inhibition of the actin cytoskeleton
- resulting →↑outflow →↓ IOP
- ↑ MMPase expression in TM cells → ECM reorganisation & Widening of empty spaces in TM
- ↑intra ocular blood flow, improve RGC survival ,promote axon regeneration
- Side effects: Transient conjunctival hyperaemia
- More compounds in this class are being investigated

Decreasing Aqueous Humor Production

- Lowering IOP by long-term reduction in aqueous humor production may not be an ideal approach to treat glaucoma
- Inhibition of aqueous humor secretion with RNA interference based gene therapeutic strategies
- Topical administration of specific siRNAs targeting carbonic anhydrase genes and alpha and beta adrenoceptors →↓ IOP in rabbits
- Advantage → the potential for producing a longer lasting effect than commercial pharmaceutical products.

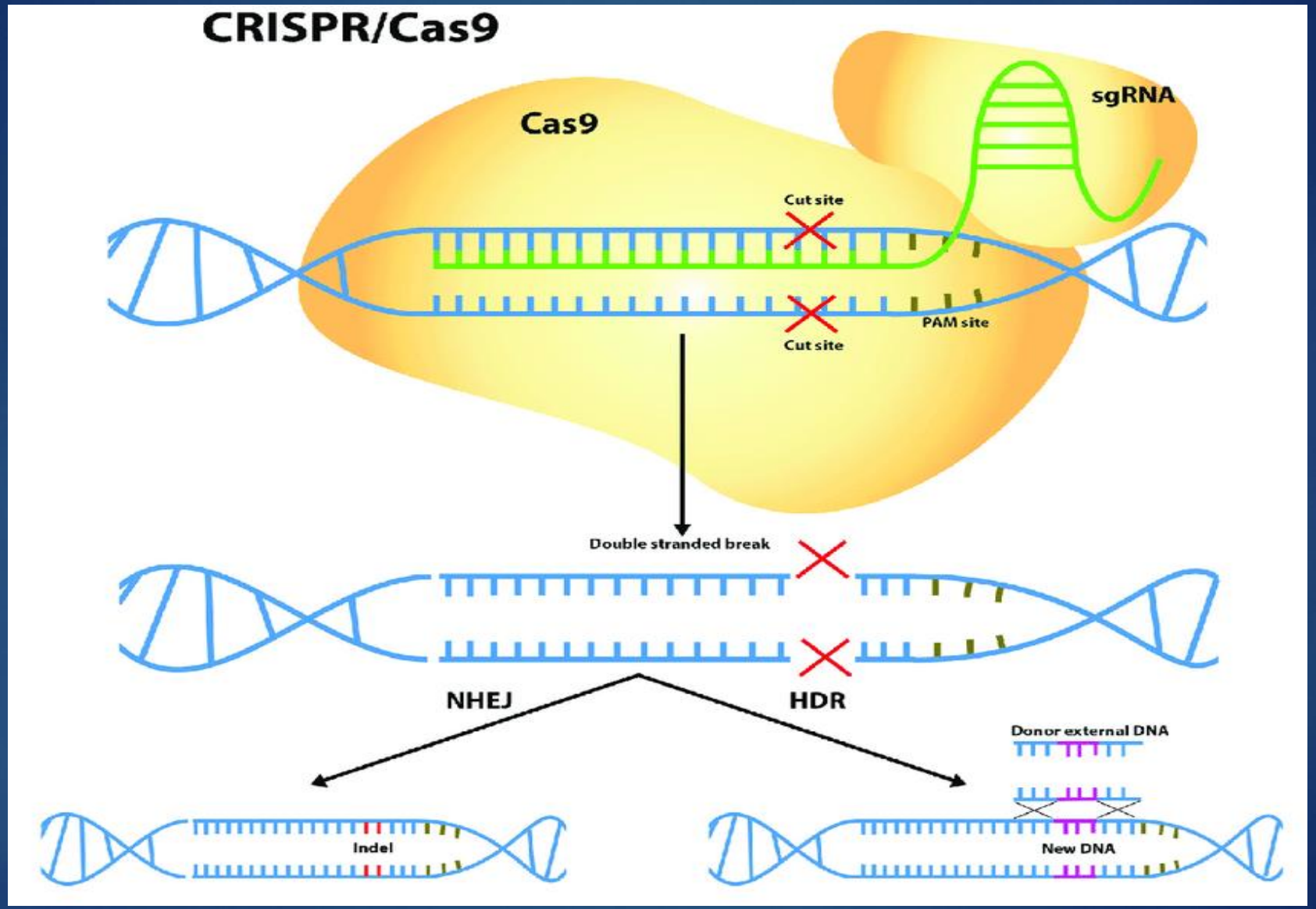
genome editing

- precise genome editing technologies Have made rapid progress
- provide the possibility of new gene therapy approaches to optic nerve diseases
  - RNA-guided nucleases (i.e., CRISPR/Cas9)
  - designer endonucleases (i.e., TALENs or ZFNs)
  - over the coming decade are likely to replace many of the current gene supplementation methods

CRISPR/Cas9

- CRISPR/Cas9-based therapies have been demonstrated for the treatment of optic diseases
- Cas9 creates site-specific double-stranded breaks in DNA
- stimulate host DNA repair mechanisms
- this system requires that two components are expressed in cells:
  - Cas9 nuclease
  - guide RNA (gRNA)

- The first 20 nucleotides of the gRNA correspond to the DNA sequence targeted
- For editing and direct Cas9 to this site using standard RNA–DNA complementarity base-pairing
- Gene breaks can be repaired through :
  - nonhomologous end joining (NHEJ)
  - creates small nucleotide insertions or deletions (indels)
  - resulting in a frameshift mutation and termination
  - homology-directed repair (HDR)
  - in the presence of a donor DNA template



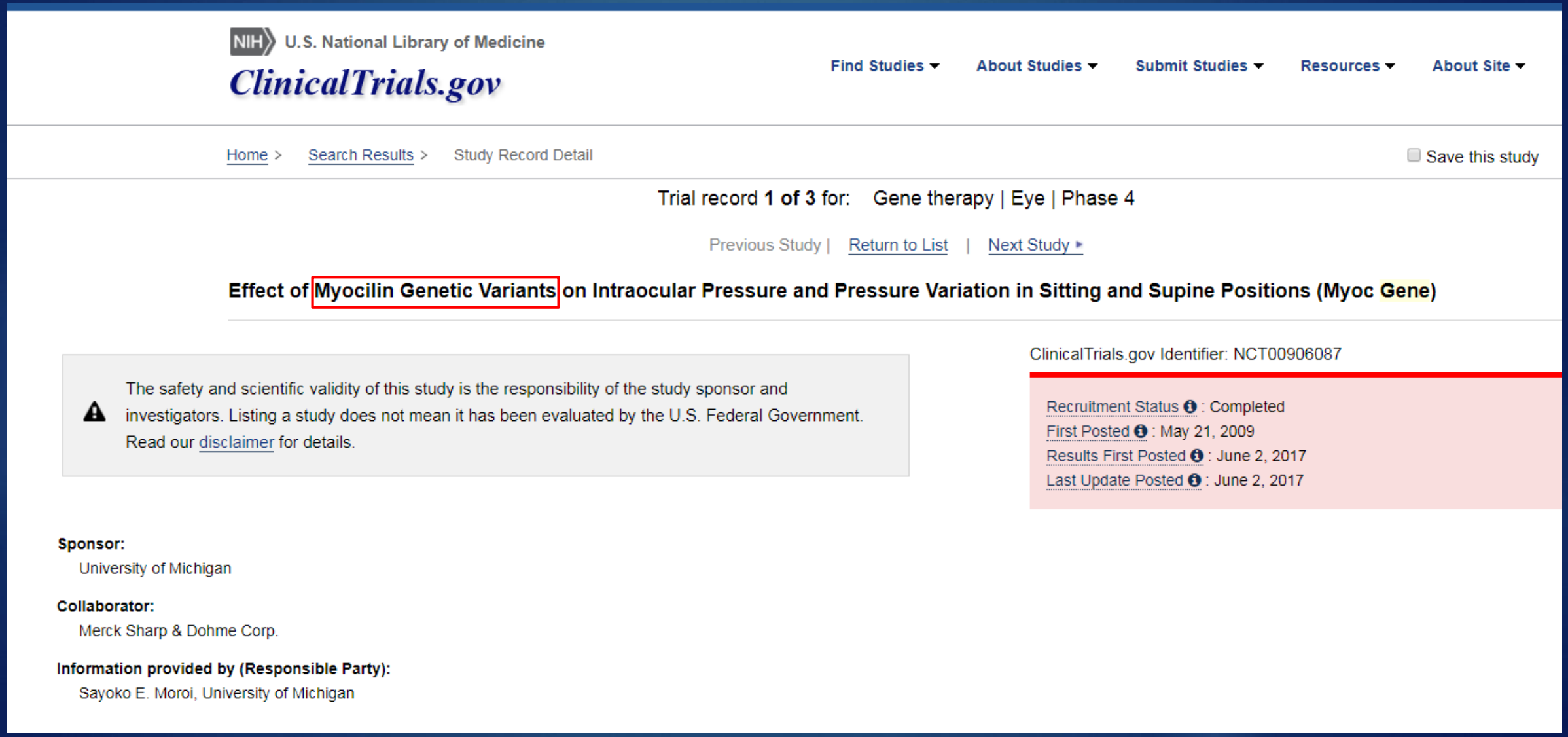
https://www.researchgate.net

- CRISPR/Cas9 can be efficiently delivered to the eye using a dual AAV system
- In a study by Hung et al, a dual AAV2 system was used
  - introduce CRISPR/Cas9 into mouse RGCs in vivo
  - achieve knockout of a YFP transgene
  - With this dual-vector system, one AAV2 delivered SpCas9
  - the other contained a single guide RNA (sgRNA) against YFP
  - achieving a knockout rate of 84% in YFP-sgRNA infected retinal cells

CRISPR/Cas9 limitation

- major concern that will need to be assessed
  - frequency of off-target activity
  - modification remains
- Possible long-term consequences may include:
  - Cas9 activation or repression, and possible epigenetic editing
- long-term AAV-mediated gene expression is beneficial for gene supplementation therapies
- CRISPR/Cas9 only requires a short period of expression

Clinical trials related to glaucoma



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Trial record **1 of 3** for: Gene therapy | Eye | Phase 4

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Effect of Myocilin Genetic Variants on Intraocular Pressure and Pressure Variation in Sitting and Supine Positions (Myoc Gene)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT00906087

Recruitment Status : Completed
First Posted : May 21, 2009
Results First Posted : June 2, 2017
Last Update Posted : June 2, 2017

Sponsor:
University of Michigan

Collaborator:
Merck Sharp & Dohme Corp.

Information provided by (Responsible Party):
Sayoko E. Moroi, University of Michigan

“Sight is the sense which is more valuable than all the rest.” So Take care of Eyes!!!

تقدیر و تشکر:

«من لم یشکر المنعم من المخلوقین لم یشکر الله عز و جل» امام رضا(ع)

- با تشکر و سپاس به در گاه باریتعالی که نخستین و بزرگترین یاریگر بندگان در آغاز و پایان هرکاریست.
- و با تقدیر و تشکر از استاد محترم جناب آقای دکتر حسین احمدپور یزدی که در گردآوری و تنظیم این مجموعه مرا راهنمایی و یاری کردند.

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Thank You
For Your Attention